Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

Seed Nitrogen Mobilization in Soybean: Effects of Seed Nitrogen Content and External Nitrogen Fertility

Erin Naegle [a]; Prachuab Kwanyuen [b]; Joseph Burton [b]; Thomas Carter [b]; Thomas Rufty [a]
[a] Department of Crop Science, North Carolina State University, Raleigh, North Carolina, USA
[b] USDA-ARS, Department of Crop Science, North Carolina State University, Raleigh, North Carolina, USA

Online Publication Date: 01 February 2008

To cite this Article: Naegle, Erin, Kwanyuen, Prachuab, Burton, Joseph, Carter, Thomas and Rufty, Thomas (2008) 'Seed Nitrogen Mobilization in Soybean: Effects of Seed Nitrogen Content and External Nitrogen Fertility', Journal of Plant Nutrition, 31:2, 367 — 379

To link to this article: DOI: 10.1080/01904160801894921
URL: http://dx.doi.org/10.1080/01904160801894921

Journal of Plant Nutrition, 31: 367–379, 2008

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904160801894921

Seed Nitrogen Mobilization in Soybean: Effects of Seed Nitrogen Content and External Nitrogen Fertility

Erin Naegle,[1] Prachuab Kwanyuen,[2] Joseph Burton,[2] Thomas Carter,[2] and Thomas Rufty[1]

[1]Department of Crop Science, North Carolina State University, Raleigh, North Carolina, USA
[2]USDA-ARS, Department of Crop Science, North Carolina State University, Raleigh, North Carolina, USA

ABSTRACT

Soybean breeding programs have developed genetic lines with relatively low seed protein, which could negatively impact early seedling growth in low fertility conditions commonly encountered in the field. In these experiments, seed protein mobilization and its regulation *in situ* in soybean lines with different seed protein levels was investigated. The results showed that rates of nitrogen (N) release from cotyledons were much lower with decreasing levels of N in seed. Patterns of proteolysis of the storage proteins glycinin and β-conglycinin and their subunits were not different, but breakdown rates were slower. Seed N release rates increased somewhat when external N was supplied to roots of the developing seedlings, suggesting the involvement of source/sink controls. The effect appeared to be down-stream from proteolysis, as rates of protein breakdown were not altered. The results indicate that low seed protein levels will lead to reduced seedling fitness in low fertility soil conditions unless fertilizer N is applied.

Keywords: Seed nitrogen release, seed nitrogen transport, soybean, storage protein degradation

INTRODUCTION

Genetic manipulation of crop species can have unintended consequences. Soybean (*Glycine max* L. Merrill) breeding programs are developing varieties with

Received 20 November 2006; accepted 15 April 2007.
Address correspondence to Thomas Rufty, Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620, USA. E-mail: tom_rufty@ncsu.edu

higher oil contents. In soybean seed, however, an inverse genetic relationship exists between oil and seed protein (Burton, 1984), i.e., increases in oil content are accompanied by proportional decreases in seed protein. The possibility of adverse consequences exists because progressive lowering of seed protein, and the associated lowering of seed nitrogen (N) reserves, may have detrimental effects for seedling development in the next generation.

Nitrogen-fixing crops like soybean are widely grown in the southeastern United States, where soils are highly weathered and have low native fertilities. Commonly, N fertilizer is not applied to soybean fields (J. Dunphy, personal communication), so the system depends on N_2 fixation to provide adequate N for achieving maximum yields (Israel, 1981). The concern arises because seedlings are dependent on N from seed reserves until N_2 fixation becomes active about 4 weeks after germination. Seedlings typically experience N stress during this developmental period, and reducing seed N reserves may contribute to a substantial decline in seedling vigor.

In a recent study, we began to evaluate seedling growth responses using soybean genotypes with a range of N contents (Naegle et al., 2005). Under low fertility conditions, it was found that lower seed N contents were associated with slower seedling growth, decreased leaf initiation and expansion, and reduced ability to respond to an external N supply. Although physiological processes had not been examined in as much detail, numerous other studies with different species also have observed that low seed N was associated with decreased seedling growth when rhizosphere fertility was low (Ries, 1971; Bulsani and Warner, 1980; Parrish and Bazzaz, 1985; Nedel et al., 1996; Hara and Toriyama, 1998; Welch, 1999; Tungate et al., 2002).

In this manuscript, additional research with soybean seedlings that examines N release from seed having different N contents is described. Specifically, attempts were made to answer the following questions: When soybean seeds have different N contents: 1) Are the patterns of seed protein degradation and N mobilization altered?; and 2), is mobilization of seed N responsive to the N status of seedlings, i.e., when seedlings are supplied with external N and the tissue N concentration is higher? The answers provide insights into the physiological changes accompanying reductions in seed protein, and their agronomic implications.

MATERIALS AND METHODS

Plant Culture

Experiments were conducted with three soybean (*Glycine max* L.) lines, NC-105, NC-112, and D68-0099 (Hartwig, 1994), which represented a wide variation in seed N content. Seeds were germinated in paper rolls moistened with 0.1 mM calcium sulfate ($CaSO_4$) and placed in a dark germination chamber

at 28°C and 98% relative humidity for approximately 72 h. Seedlings with radicles 8 to 12 cm long were moved into 50-L continuous-flow hydroponics systems for 27 d. Treatment solutions with and without N were randomly assigned to eight hydroponics units (four per N treatment), and seedlings of each line were randomly distributed among the units. The units were located in a walk-in growth chamber in the North Carolina State University Phytotron (Raleigh, NC) programmed for a day/night temperature regime of 26/22°C. Plants were exposed to a nine-hour light period with cool white fluorescent and incandescent light, providing a photosynthetic photon flux density (PPFD) of 550 ± 50 μmol $m^{-2}s^{-1}$. A three-hour night interruption with incandescent light of insignificant PPFD (30.5 ± 3.4 μmol $m^{-2}s^{-1}$), provided sufficient photomorphogenic irradiance, 11 ± 1 W m^{-2}, to repress flowering.

The nutrient solution temperature was maintained at 24 ± 0.5°C and pH at 6.0 ± 0.2 with automated monitoring and addition of potassium hydroxide (KOH) (0.01 mM) and sulfuric acid (H_2SO_4) (0.01 mM). The complete nutrient solution contained: 600 μM potassium nitrate (KNO_3), 200 μM monopotassium phosphate (KH_2PO_4), 300 μM magnesium sulfate ($MgSO_4$), 800 μM calcium sulfate ($CaSO_4$), 19 μM boric acid (H_3BO_3), 3.7 μM manganese chloride ($MnCl_2$), 317 nM zinc sulfate ($ZnSO_4$), 132 nM copper sulfate ($CuSO_4$), 50 nM molybdic acid (H_2MoO_4), and 35.8 μM iron (Fe) as Fe-Sequestrene. When plants were grown without an external N source, KNO_3 was replaced with 300 μM potassium sulfate (K_2SO_4). Nutrients were monitored and adjusted so that depletion was less than 30% of the initial solution concentrations.

Seedlings were harvested periodically during the 3-day germination period and the subsequent 24 d in hydroponics. It was found that most of the changes in biochemical parameters occurred by day 21, so measurements of biochemical parameters stopped at that time. At each harvest, eight plants per line and N treatment were sampled, two plants from each of four chambers. Plants were immediately divided into shoots, roots, and cotyledons. Cotyledons were kept on ice, and within 30 min frozen at −80°C. Cotyledons were freeze-dried, weighed, and ground to a fine powder. Shoots and roots were dried in an oven at 55°C, weighed, and ground. Nitrogen content was measured using an elemental analyzer (Model Flash EA 1112, ThermoQuest, Rodano, Milano, Italy).

Soluble Protein Extraction and Separation

Ground cotyledon tissues from the eight plants at each harvest were combined, and 0.35 g of tissue were placed into a 15 mL test tube. Soluble protein was extracted with 0.2 M Tris-HCl buffer, pH 8.0, containing 0.1 M β-mercaptoethanol in a 1:20 w/v (Kwanyuen and Wilson, 2000). The mixture was vigorously stirred for one hour at room temperature and centrifuged at 10,000 g for 10 min at 4°C. An aliquot of the supernatant was combined with an equal volume of solution containing 5% SDS and 0.1 M β-mercaptoethanol and placed in a boiling water

bath for 10 min to dissociate soluble proteins. Bromophenol blue and glycerol were added to the dissociated proteins for a final concentration of 0.025% and 10%, respectively. Samples were frozen until used for electrophoresis.

Dissociated proteins were separated utilizing a Bio-Rad (Richmond, CA) Protean II vertical slab gel apparatus (Chua, 1980). The gel dimensions were $14 \times 16 \times 0.15$ cm with a linear gradient of 10 to 20% polyacrylamide. Protein was loaded into every other well to avoid cross contamination between lanes and maximize clarity for scanning densitometry. Gels were loaded with 80–100 μg of protein per well. Electrophoresis was conducted at room temperature for 14–16 h at 7.5 mA/gel. Protein concentrations in crude extracts were determined using the Bradford protein assay (Bradford, 1976). Protein content on a per plant basis or per cotyledonary pair was estimated by multiplying protein concentration by the extraction volume and the ratio of the averaged weight of cotyledons to the extraction weight. Nitrogen content within protein was calculated using a conversion factor of 5.75. Nitrogen content of crude protein extract was measured by flash combustion N analysis on dried extract samples, with the N in the Tris-HCl buffer measured and subtracted out. Soluble protein buffer extraction of the tissue recovered 95.2% $\pm$ 1.65 of the total N.

Gels were fixed for 30 min in a solution of 40% (v/v) methanol and 10% (v/v) acetic acid, and stained with 0.25% (w/v) Coomassie Brilliant Blue in 40% methanol and 10% acetic acid. Gels were destained with the methanol, acetic acid solution until the background of the gel was nearly clear of blue dye. This consisted of changing the destaining solution every 2 h at least four times. Fixation, staining, and destaining of gels occurred on an orbit shaker at room temperature. After destaining, gels were sandwiched between cellophane sheets, and dried in a Bio-Rad (Richmond, CA) GelAir dryer.

Protein Quantification

Gels were scanned with a Molecular Dynamics Personal Densitometer SI (Sunnyvale, CA) equipped with a HeNe laser light source. Volume integration was performed using ImageQuant software. Background absorbance was subtracted from total absorbance of protein bands. Relative amounts of identified bands were expressed as percent of total protein in a lane. Bands identified as soybean storage protein subunits were α', α, and β for β-conglycinin, and A3, acidic, and basic for glycinin. Concentrations of storage protein subunits were multiplied by the calculated total protein to estimate protein subunit content (mg protein plant^{-1}).

Amino Acid Quantification

Due to its binding properties, Coomassie Brilliant Blue of the Bradford assay leaves free amino acids undetected (Compton and Jones, 1985); therefore, free amino acids in the cotyledons were quantified separately from the proteins and

then combined to estimate the size of the amino acid pool. The concentration of free amino acids in the crude extract was determined by reverse-phase high-performance liquid chromatography (Heinrikson and Meredith, 1984). Hydrolysis was not performed prior to derivitization with phenylisothiocyanate, in order to keep proteins and peptides intact. Derivitized samples were run in duplicate with an internal standard of methionine sulfone. After adjusting for the internal standard, sample amino acids were quantified based on protein hydrolysate standard (Pierce, Rockford, IL), and through a series of calculations expressed as mg N cotyledon pair^{-1}.

RESULTS

The three soybean lines had several-fold differences in seed N contents (Table 1), ranging from 4.5 to 14.6 mg N per seed. When seedlings were germinated and grown in the absence of external N, dry weight accumulation was different among lines, and growth differences were positively related to seed N content. Compared with +N controls, seedlings from all three of the soybean lines clearly were smaller and thus N limited. The growth effects were not due to inherent growth differences among the soybean lines, because the three lines grew similarly when N was present in the solutions.

Seed N Mobilization

Cotyledonary N content decreased exponentially (Figure 1A; Naegle et al., 2005). Amounts of N released reflected differences in the initial seed N content. The level of N in the cotyledons approached zero by about day 21 in all treatments, so seed N release rates were slower with lower seed N. Lower soluble protein levels also were associated with slower degradation rates (i.e.,

Table 1
Differences in seed parameters of three soybean lines and dry weights of seedlings after 27 days of growth. Seed N content is the product of weight and N concentration. Numbers in parentheses are standard error of the mean of 8 seeds or plants

Line	Avg. weight (mg seed^{-1})	N concentration (%N)	Avg. N content (mg seed^{-1})	Plant dry weights at 27 days (mg plant^{-1})	
				+N	−N
NC-105	195.1 (5.1)	7.5 (0.1)	14.6 (0.4)	1920.7 (148.8)	450.5 (61.6)
NC-112	125.8 (2.3)	7.2 (0.2)	9.0 (0.2)	1897.0 (83.4)	233.8 (12.5)
D68-0099	90.1 (4.0)	4.7 (0.5)	4.5 (0.5)	1879.0 (105.4)	135.6 (17.1)

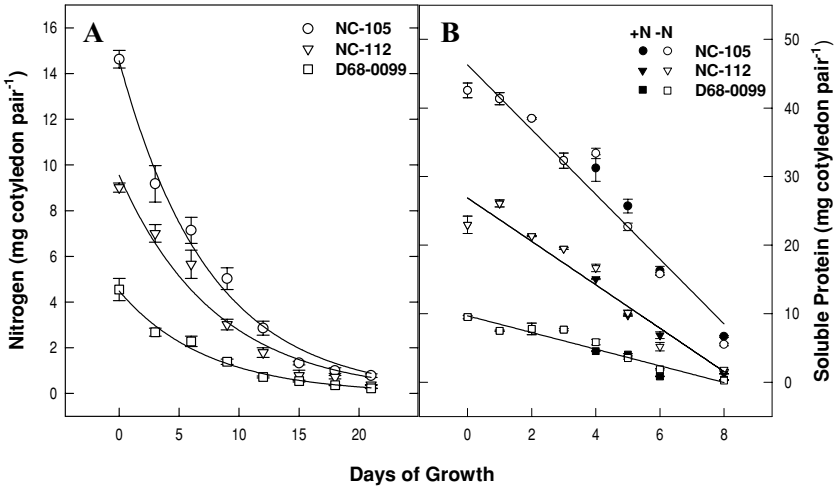

Figure 1. Changes in the N content (A) and soluble protein (B) in cotyledons of 3 genotypes of soybean supplied or deprived of external N in solution. The N treatments are plotted as a single line for each genotype. Only −N symbols are visible where symbols overlap. Vertical bars represent mean standard error (n = 3).

slopes of the plotted lines, Figure1B). With + and −N treatments combined, the degradation rates of the three lines were 4.7 ± 0.042, 3.2 ± 0.33, and 1.3 ± 0.21 mg N plant^{-1} day^{-1} for NC-105, NC-112, and D68-0099, respectively. Soluble protein levels decreased to minimal amounts within about 8 d, so protein degraded more rapidly than N was released from the cotyledons. There were no significant differences in the soluble protein degradation rates between the + and −N treatments within a genotype.

The data for cotyledon N shown in Figure 1A were natural log transformed to delineate differences in N release rates in the + and −N treatments (Table 2; Figure 2). The plots indicated that seed N was depleted more rapidly in −N than +N treatments in all three of the soybean lines. More rapid mobilization under

Table 2
Slopes of linear transformations of cotyledonary N data shown in Figure 2. All $r^2 > 0.95$; p values result from F-tests conducted on +N vs. −N within a soybean line. Numbers in parentheses represent standard error of the mean

Line	+N	−N	+ N vs −N
NC-105	−0.132 (0.004)	−0.187 (0.018)	$p < 0.001$
NC-112	−0.132 (0.009)	−0.194 (0.017)	$p < 0.001$
D68-0099	−0.124 (0.007)	−0.170 (0.001)	$p < 0.001$

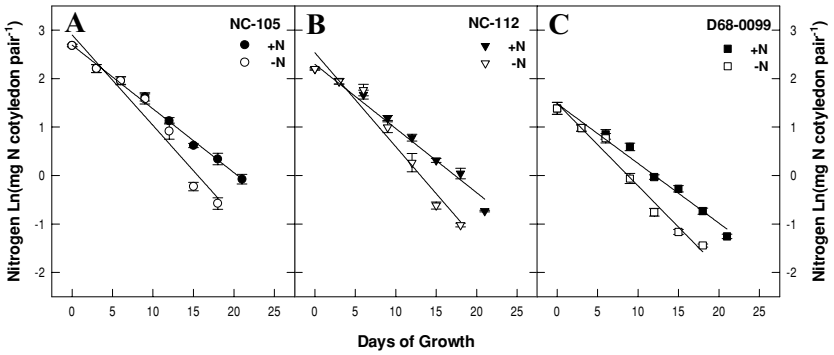

Figure 2. Natural log transformed N levels in cotyledons of seedlings supplied with or deprived of external N. All r^2 values (>.95) were highly significant, and slopes between N treatments for each genotype were all significantly different (see Table 2; $p < 0.001$).

−N could be detected with NC-105 on day 15, but with NC-112 on 12, and with D68-0099 on day 9. Thus, more rapid N mobilization was always present in seedlings without external N and the appearance of a higher mobilization rate occurred sooner when less N was present in the seed.

The primary storage proteins glycinin and β-conglycinin generally account for 55 to 80% of the N in soybean seed (Murphy, 1984; Murphy and Resurreccion, 1984). Their patterns of degradation were similar among the soybean lines, and no differences could be detected in the + and − N treatments (Figure 3). Different levels of glycinin and β-conglycinin were present at the beginning of the experiment, and the time required for complete degradation was similar, with glycinin degraded by day 6 and β-conglycinin by day 4. Thus, as with total soluble protein, storage protein breakdown rates were slower with lower protein among the soybean lines.

The degradation patterns for the subunits of glycinin and β-conglycinin in the three soybean lines were similar to those observed previously (Figure 4; Wilson et al., 1986, 1988). The acidic subunits of glycinin depleted faster than basic subunits, approaching zero within about 4 d compared to day 6 (Figures 4A, 4B, and 4C). With β-conglycinin, α subunits were present in much greater amounts than the β subunits and the α subunits degraded very rapidly (Figures 4D, 4E, and 4F). Among the soybean lines, the time to complete degradation again was similar, so breakdown rates were positively correlated with the amount of subunit protein present. No differences could be detected in subunit degradation in the + and −N treatments. It should be noted that the β subunit was only faintly detectable in D68-0099. It is often absent or present in very small quantities in this soybean line with its low seed N status (Ohtake et al., 1997).

Amino acids, the final products of protein degradation, evidently are the primary form of N transported from the cotyledons into the developing seedling.

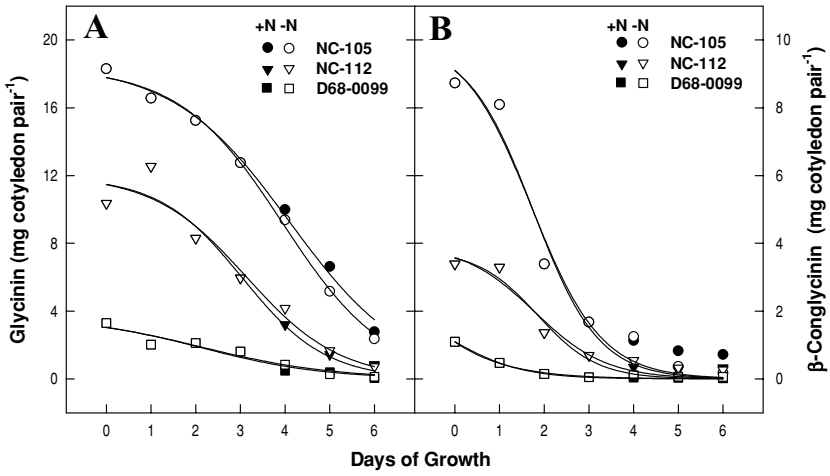

Figure 3. Degradation of storage proteins glycinin (A) and β-conglycinin (B) over the first six days of growth. Note differences in vertical scale between panels. Only $-$N symbols are visible where symbols overlap.

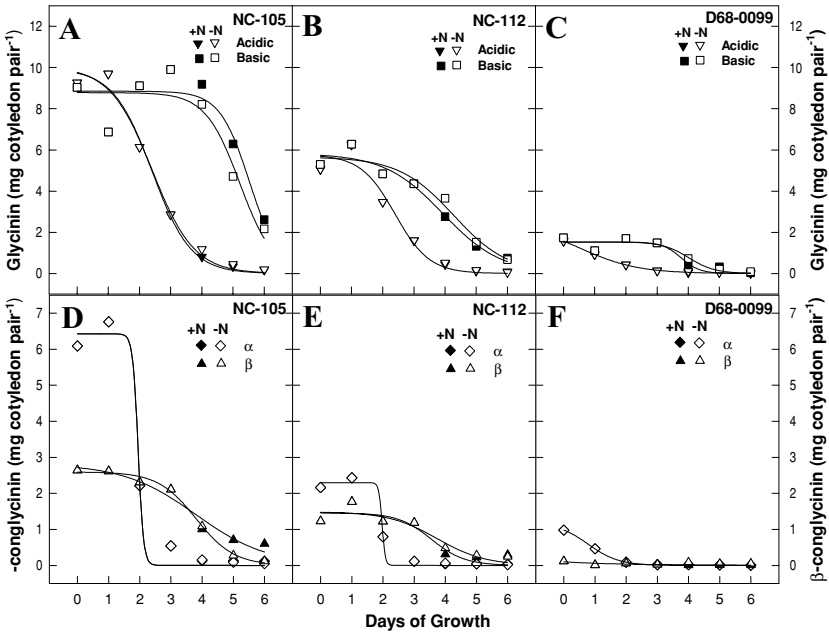

Figure 4. Degradation of the subunits of the glycinin and β-conglycinin storage proteins over time. Data for $+$ and $-$N treatments could not be separated statistically and were plotted as a single line. Only $-$N symbols are visible where symbols overlap.

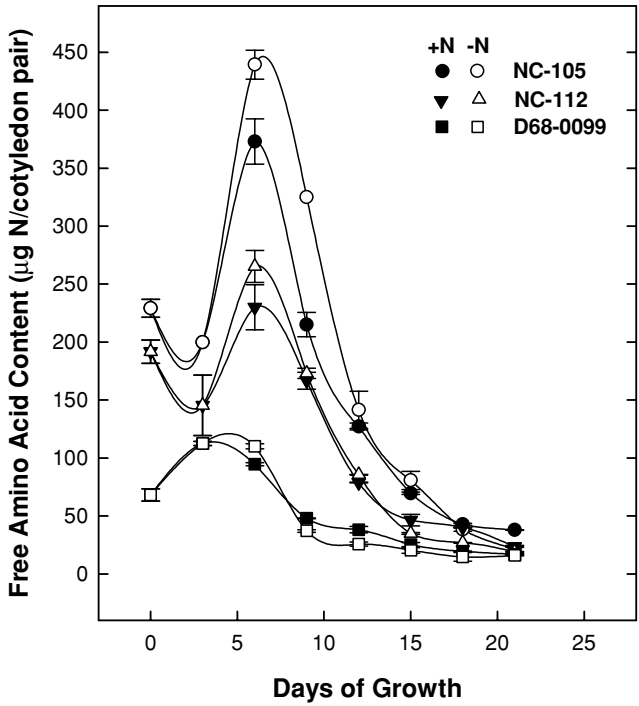

Figure 5. Total content of free amino acids of the three soybean genotypes with varying seed N contents. Only −N symbols are visible where symbols overlap.

The total free amino acid pools within the cotyledons were relatively low compared to the total amount of protein present in the cotyledons initially (compare plots and y-axis of Figures 5 and 1b), which might be expected of an intermediate pool where substrate is being efficiently transported to vegetative, sink tissues. Amino acid levels increased until day 6 and then declined (Figure 5). Thus, the maximum peak in amino acid content coincided with the end of the protein breakdown phase. At many of the sampling intervals, and certainly on day 6, higher amino acid levels were present in cotyledonary tissues of −N plants.

DISCUSSION

The initial question being addressed in these experiments was how N mobilization from seed changed when the seed N content was lower. While biochemistry of protein degradation in soybean seed has been described previously, many aspects of regulation of degradation *in situ* have not been examined. The patterns of seed protein degradation observed in these studies were similar to those described in the literature (McAlister and Krober, 1951; Murphy,1984; Mur-

phy and Resurreccion, 1984; Nielsen, 1985; Wilson et al., 1986, 1988; Muntz, 1996). Results, however, showed that protein degradation occurred over about the same time frame, regardless of the amount of protein present in the three soybean lines. Thus, breakdown rates were proportional to the amounts of protein initially present. This can be seen in the plots of individual proteins and their subunits, disappearance of soluble protein, and heightened amounts in the amino acid transport pool. Greater rates of seed protein degradation at higher seed N levels imply that the substrate specific proteases responsible for storage protein degradation (Wilson et al., 1986; Qi et al., 1992; Muntz, 1996) were present in greater abundance with increasing protein content or had higher activities.

Another question in these experiments was whether seed N mobilization responded to the N status of the developing seedling. Theoretically, the physiological impact of supplying N to seedlings during cotyledon N mobilization is that sink demand for N is reduced. The natural log plots of seed N indicated that depriving seedlings of an external N source (i.e., higher sink demand) increased the rate of N release from the cotyledons in each of the soybean lines (Figure 2). Complete depletion of the cotyledon N pool was sped up by several d. It seemed that the −N regulatory effect occurred downstream from protein disassembling, as no change could be detected in amounts of soluble protein or degradation of individual storage proteins and their subunits. A degree of uncertainty exists on this point, because seedlings were exposed to N treatment solutions on day 3 after germination began and protein degradation was well underway by that time.

The exact processes regulating N transport from cotyledonary tissue are being investigated intensively (refer to Miranda et al., 2003; Stacy et al., 2006) and much is still unknown. That being said, the responsiveness of N release to changes in vegetative sink demand resembles that of the feedback systems regulating entry of nitrate and ammonium into vegetative tissues through uptake processes in the root (Imsande and Touraine, 1994; Glass, 2003). Borrowing from the root feedback models, one might speculate that amino acid and perhaps peptide transporters (Stacey et al., 2002, 2006) in the cotyledons are up-regulated when levels of amino acids or nitrate in adjoining vegetative tissues are low.

It should be emphasized that although source/sink effects seem to be present in cotyledonary N transport, the major factor controlling N release into vegetative tissues is the N content of the seed. The seed N unloading process functions primarily as a 'feed-forward' system with the great majority of the N available in seed moved into vegetative tissues within a particular time frame. The source/sink effects offset the time window only by several days. We are aware of no studies that offer directly comparable results as those generated here. In a previous study with *Lupinus*, slower N mobilization out of cotyledons was observed when plants were deprived of N (Hocking, 1980). The conflicting results, however, may be due to differences in methodology. Only distilled water

was supplied to the *Lupinus* seedlings, so they were deprived of all nutrition. The seedlings may have experienced multiple stresses that would have decreased the growth potential and 'demand' for N.

An important result of reduced release of N from the seed/cotyledon is that seedling fitness would be decreased. At this early stage of development, seedlings do not possess the degree of 'morphological plasticity' that is present later on. As shown previously, slower seedling growth is associated with reduced leaf canopy development, but not proportionate enhancement of root growth (Naegle et al., 2005). The adjustment in shoot and root growth ratios is considered to be a key acclimation strategy for survival and competitiveness in low N environments (Chapin, 1988, 1991; Rufty, 1997). Also, decreased vigor has been associated with lower nodulation (Smith and Ellis, 1980), and thus an impaired ability to acquire N through another physiological process. The end result is that low seed N soybean lines will produce seedlings that are less efficient at acquiring resources from the soil and less competitive with weeds. A logical conclusion that can be drawn from these experiments is that when growing high oil, low protein lines, N fertilizer should be applied at the time of planting to minimize the negative effects of the limited internal N supply.

REFERENCES

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.

Bulsani, E. A., and R. L. Warner. 1980. Seed protein and nitrogen effects upon seedling vigor in wheat. *Agronomy Journal* 72: 657–666.

Burton, J. W. 1984. Breeding soybeans for improved protein quantity and quality. In *Proceedings of the World Soybean Research Conference III*. Ed. R. Shibles. pp. 361–367. Westview Press, Inc. Boulder, CO.

Chapin, F. S. 1991. Integrated responses of plants to stress. *Bioscience* 41: 27–36.

Chapin, F. S. 1988. Ecological aspects of plant mineral nutrition. *Advances in Plant Nutrition* 3: 161–191.

Chua, N. H. 1980. Electrophoretic analysis of chloroplast proteins. *Methods in Enzymology* 69: 434–446.

Compton, S. J., and C. G. Jones. 1985. Mechanism of dye response and interference in the Bradford protein assay. *Analytical Biochemistry* 151: 369–374.

Glass, A. D. M. 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. *Critical Revenues in Plant Science* 22: 453–470.

Hara, Y., and K. Toriyama. 1998. Seed nitrogen accelerates the rates of germination, emergence, and establishment of rice plants. *Soil Science and Plant Nutrition* 44: 359–366.

Hartwig, E. E. 1994. Registration of near-isogenic soybean germplasm lines D68-0099 and D68-0102, differing in ability to form nodules. *Crop Science* 34: 822.

Heinrikson, L., and S. C. Meredith. 1984. Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Analytical Biochemistry* 136: 65–74.

Hocking, P. J. 1980. Redistribution of nutrient elements from cotyledons of two species of annual legumes during germination and seedling growth. *Annals of Botany* 45: 83–396.

Imsande, J., and B. Touraine. 1994. Demand and the regulation of nitrate uptake. *Plant Physiology* 105: 3–7.

Israel, D. W. 1981. Cultivar and *Rhizobium* strain effects on nitrogen fixation and remobilization by soybeans. *Agronomy Journal* 73: 509–516.

Kwanyuen, P., and R. F. Wilson. 2000. Optimization of Coomassie staining for quantitative densitometry of soybean storage proteins in gradient gel electrophoresis. *Journal of the American Oil Chemists Society* 77: 1251–1254.

McAlister, D. F., and O. A. Krober. 1951. Translocations of food reserves from soybean cotyledons and their influence on the development of the plant. *Plant Physiology* 26: 525–538.

Miranda, M., L. Borisjuk, A. Tewes, D. Dietrich, D. Rentsch, H. Weber, and U. Wobus. 2003. Peptide and amino acid transporters are differentially regulated during seed development and germination in faba bean. *Plant Physiology* 132: 1959–1960.

Muntz, K. 1996. Protease and proteolytic cleavage of storage proteins in developing and germinating dicotyledonous seeds. *Journal of Experimental Botany* 47: 605–622.

Murphy, P. A. 1984. Structural characteristics of soybean glycinin and β-conglycinin. In *Proceedings of the World Soybean Research Conference III*. Edited by Shibles, R. pp. 143–151. Westview Press, Boulder.

Murphy, P. A., and A. Resurreccion. 1984. Varietal and environmental differences in soybean glycinin and β-conglycinin content. *Journal of Agricultural Food Chemistry* 32: 911–915.

Naegle, E. R., J. W. Burton, T. E. Carter, and T. W. Rufty. 2005. Influence of seed nitrogen content on early leaf development and ability to recover from nitrogen stress. *Plant and Soil* 271: 329–340.

Nielsen, N. C. 1985. The structure and complexity of the 11s polypeptides in soybeans. *Journal of American Oil Chemists Society* 62: 1680–1686.

Nedel, J. L., S. E. Ullrich, and W. L. Pan. 1996. Effect of seed size and protein content and N application timing on seedling vigor and grain yield of barley. *Pesquisa Agropecuaria Brasileira* 31: 113–119.

Ohtake, N., S. Yamada, M. Suzuki, N. Takahashi, Y. Takahashi, T. Chinushi, and T. Ohyama. 1997. Regulation of accumulation of β-subunit of

β-conglycinin in soybean seeds by nitrogen. *Soil Science and Plant Nutrition* 43: 247–253.

Parrish, J. A. D., and F. A. Bazzaz. 1985. Nutrient content of *Abutilon theophrasti* seeds and the competitive ability of the resulting plants. *Oecologia* 65: 247–251.

Qi, K., K. A. Wilson, and A. L. Tan-Wilson. 1992. Characterization of the major protease involved in the soybean β-conglycinin storage protein mobilization. *Plant Physiology* 99: 725–733.

Ries, S. K. 1971. The relationship of protein content and size of bean seed with growth and yield. *Journal of the American Society of Horticultural Science* 96: 557–560.

Rufty, T. W. 1997. Probing the carbon and nitrogen interaction: a whole plant perspective. In *A Molecular Approach to Primary Metabolism in Higher Plants*. Eds. CH Foyer, P Quick, pp. 255–273. London, U.K.: Taylor and Francis.

Stacey, G., S. Koh, C. Granger, and J. M. Becker. 2002. Peptide transport in plants. *Trends in Plant Science* 7: 257–263.

Stacy, M. G., H. Osawa, A. Patel, W. Gassmann, and G. Stacey. 2006. Expression analyses of *Arabidopsis* oligopeptide transporters during seed germination, vegetative growth and reproduction. *Planta* 223 (2): 291–305.

Smith, R. S., and M. A. Ellis. 1980. Soybean nodulation as influenced by seedling vigor. *Agronomy Journal* 72: 605–608.

Tungate, K. D., D. J. Susko, and T. W. Rufty. 2002. Reproduction and offspring competitiveness of *Senna obtusifolia* are influenced by nutrient availability. *New Phytologist* 154: 661–669.

Welch, R. M. 1999. Importance of seed mineral nutrient reserves in crop growth and development. In *Mineral Nutrition of Crops*. Ed. Z Rengel. pp. 205–226. New York: Food Products Press.

Wilson, K. A., B. R. Rightmire, J. C. Chen, and A. L. Tan–Wilson. 1986. Differential proteolysis of glycinin and β-conglycinin polypeptides during soybean germination and seedling growth. *Plant Physiology* 82: 71–76.

Wilson, K. A., G. Papastoitsis, P. Hartl, and A. L. Tan-Wilson. 1988. Survey of the proteolytic activities degrading the Kunitz trypsin inhibitor and glycinin in germinating soybeans (*Glycine max*). *Plant Physiology* 88: 355–360.